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Microhypsibiidae, new family of eutardigrades, and description of the new genus *Fractonotus*

(Tardigrada)

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Pilato, G. (1998): Microhypsibiidae, new family of eutardigrades, and description of the new genus *Fractonotus* (Tardigrada). – Spixiana **21/2**: 129-134

The structure of the claws of *Microhypsibius* type, of *Calohypsibius* type, of *Eohypsibius* type and of *Hypsibius* type is analyzed. The proposed new family Microhypsibiidae is described. Two genera are assigned to it: *Microhypsibius* Thulin, 1928 and *Fractonotus*, gen. nov. The latter differs from the former in the presence of a pair of elliptical organs on the head, and in characteristics of the bucco-pharyngeal apparatus.

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Introduction

The claws of the eutardigrades, in relation to the reciprocal position of the basal portion, of the main branch and the secondary branch, and to the type of connexion between the branches, can have different structure (i.e. it can be different the model according to which the claw is built). The claws of each model can have different shape.

Pilato (1969) stressed the significance of the claw structure which is considered a conservative character, phylogenetically very significant (Pilato 1969, 1975, 1982, Bertolani 1981, Bertolani & Kristensen 1987); the families are distinguished from one another on the basis of the symmetric or asymmetric arrangement of the diploclaws with respect to the median plane of the legs, and/or on the basis of the claw structure.

Thulin (1928), describing the genus *Microhypsibius*, considered its claws similar to those of the genus *Calohypsibius* Thulin, 1928. Marcus (1929) did not consider *Microhypsibius* a valid genus and transferred *Microhypsibius truncatus* Thulin, 1928 to *Calohypsibius*, considered by him as a subgenus of *Hypsibius*. Pilato (1969) defined more precisely the structure of the claws of *Calohypsibius* type, gave back generic rank to *Calohypsibius* and instituted the family Calohypsibiidae to which he ascribed the eutardigrades having claws of *Calohypsibius* type.

Since he could not examine any species attributed to *Microhypsibius*, Pilato (1969) did not express any opinion about that genus. Kristensen (1982) revalidated the genus *Microhypsibius* Thulin, 1928 and described two new species: *Microhypsibius bertolanii* and *M. minimus*. Kristensen, in agreement with Thulin, considered the claws of *Microhypsibius* similar to those of *Calohypsibius*, therefore he ascribed the genus *Microhypsibius* to the family Calohypsibiidae. Pilato (1982) suggested that *Microhypsibius* does not have claws of *Calohypsibius* type and that this genus cannot be ascribed to the family Calohypsibiidae. Bertolani & Kristensen (1987) stressed the differences between the claws of *Microhypsibius* (which they described as small, rigid, with a common basal segment and without clear internal limits between the three parts) and those of *Calohypsibius* type. They removed *Microhypsibius* from the family Calohypsibiidae and transferred it to the family Hypsibiidae. Ito (1991) described *Microhypsibius japonicus* but considered it a member of the family Calohypsibiidae.

Material

Specimens of *Microhypsibius truncatus*, *M. bertolanii*, *M. minimus*, *Calohypsibius ornatus*, *Calohypsibius caelatus* were examined, and the claws of *Microhypsibius* were compared with those of the Calohypsibiidae, Hypsibiidae and Eohypsibiidae.

Results

In the claws of *Calohypsibius* type (Figs 1a, b) the secondary branch is rigidly joined to the primary branch from the base of the claw (the suture is clearly visible). The basal portion of the claw is therefore wide, stumpy, without a narrow "peduncular" portion. Moreover, in all known species of Calohypsibiidae the two diploclaws of each leg are similar to one another in shape and size; the claws are small (sometimes extremely small); the secondary branch in many cases is reduced.

In the claws of Eohypsibiidae (Fig. 1c) the three, clearly distinguishable, portions of the claw (basal portion, secondary branch and main branch) are sequentially arranged in the above mentioned order and distinct from one another by a septum. The internal claws can rotate on their bases, sometimes simulating the symmetric arrangement of the claws with respect to the median plane of the leg.

In the claws of the Hypsibiidae (Fig. 1d) a basal portion, often long and narrow, is present, continuous with the secondary branch; the primary branch is joined to the secondary branch through a flexible connection. In some cases this connection is very slightly sclerified: in a few cases (hind legs of *Eremobiotus* and, perhaps, some species of *Isohypsibius*) it is highly sclerified so that the connection appears to be rigid. Moreover, in the species having claws of Hypsibiidae type, the two claws of each leg are different in shape and size from one another. None of the known species of Hypsibiidae (more than 220) has claws with clearly reduced secondary branch.

The claws of *Microhypsius* type, like those of the Hypsibiidae, have a fairly long, narrow, basal portion (Fig. 1e). Differently from the claws of the Hypsibiidae, this portion seems to be continuous with the primary branch; the secondary branch is rigidly joined to the primary branch, therefore there is not a flexible connexion between the two branches. The two claws of each leg are slightly different in shape and size from one another.

The claws of *Microhypsibius* appear similar in shape to those of the Hypsibiidae, but the structure is different and therefore one can conclude that the species having claws of *Microhypsibius* type belong to a phyletic line about equidistant from that of the Hypsibiidae and from those of the Calohypsibiidae and of the Eohypsibiidae.

The claws of *Microhypsibius* type, like those of the Hypsibiidae and Eohypsibiidae, but unlike those of the Calohypsibiidae, have a narrow, "peduncular", basal portion. Like in the claws of the Calohypsibiidae but unlike those of the Hypsibiidae and Eohypsibiidae, the basal portion seems to be continuous with the primary branch. The claws of *Microhypsibius* type, like those of the Calohypsibiidae and the Eohypsibiidae, but unlike those of the Hypsibiidae, have the secondary and the primary branches rigidly joined to one another. Unlike the claws of the Eohypsibiidae, in the claws of *Microhypsibius* type, the basal portion, the secondary branch and the primary branch are not sequentially arranged, and the internal claws cannot rotate on their bases.

It seems justified to conclude that the species having claws of *Microhypsibius* type might belong to an evolutionary line to which the value of family or of subfamily of Hypsibiidae, could be attributed. I consider the institution of a new family (Microhypsibiidae) better justified than the institution of a new subfamily (within the family Hypsibiidae), both because the significance of the structural differences of the claws, and because the three extant subfamilies of Hypsibiidae (Hypsibiinae, Itaquasconinae and Diphasconinae) do not differ from each other in the structure of the claws. The proposed new family, named Microhypsibiidae, can be defined as follows:



Fig. 1. Claws. **a**, **b**. *Calohypsibius* type. **c**. *Eohypsibius* type. **d**. Hypsibiidae type. **e**. *Microhypsibius* type (*Fractonotus caelatus*). bp: basal portion; pb: primary branch; sb: secondary branch. Scale bar = $10 \mu m$.

Microhypsibiidae, fam. nov.

Diagnosis. Eutardigrades having claws arranged asymmetrically with respect to the median plane of the legs. Claws of *Microhypsibius* type: the claws have a narrow basal portion continuous with the primary branch; the secondary branch is rigidly joined to the primary branch. The internal claws cannot rotate on their bases.

Two genera belong to the new family: *Microhypsibius* Thulin, 1928 and *Fractonotus*, gen. nov. (described below).

Microhypsibius Thulin, 1928

All known species having claws of *Microhypsibius* type are presently ascribed to the genus *Microhypsibius* (*M. truncatus* Thulin, 1928; *M. bertolanii* Kristensen, 1982; *M. minimus* Kristensen, 1982 and *M. japonicus* Ito, 1991).

Concerning the bucco-pharyngeal apparatus, Kristensen (1982) stated that *M. truncatus* and *M. minimus* have apophyses for the insertion of the stylet muscles in the shape of "ridges", whereas *M. bertolanii* has a thin strengthening bar (or ventral lamina).

In examining a paratype specimen in perfectly lateral view, I noted that *M. bertolanii* has no ventral lamina, and has apophyses for the insertion of the stylet muscles similar to those of *M. truncatus* and *M. minimus*. I noted also that in all these species, the apophyses for the insertion of the stylet muscles are not simple ridges. The ventral apophysis (Fig. 2b) is a well developed ridge having, at $\frac{2}{3}$ of its length, a prominence in the shape of a blunt hook. The dorsal apophysis can be interpreted as a ridge split into two portions. The anterior portion forms, caudally, a little hook which is similar in shape to the hooks of *Hypsibius*, and which, therefore, can be defined a "semilunar hook". The caudal portion appears as a short thickening, gradually decreasing in height. The apex of the dorsal hook is very near to the wall of the buccal tube and therefore, when the specimens are not in lateral position, the hook is not clearly distinguishable and the apophysis seems in shape of a simple ridge.

The subdivision of the apophyses for the insertion of the stylet muscles into two portions is a common feature in some evolutionary lines of eutardigrades: it is evident in *Ramazzottius* (Binda & Pilato, 1986), in *Mixibius* (Pilato, 1992) and in *Acutuncus* (Pilato, 1997). Though not stressed in the literature, I consider that the extant apophyses for the insertion of the stylet muscles are corresponding to the anterior portion of primarily long ridges like those present in *Ramajendas* (Pilato & Binda, 1990). On the basis of the morphology of the apophyses, one can assume that:

- a. in many evolutionary lines of eutardigrades, the primary ridges have split into two portions (the anterior formed the extant apophysis for the insertion of the stylet muscles, the caudal portion can be more or less reduced or completely absent);
- b. in other evolutionary lines the primary ridges have undergone the reduction of their caudal portion without any breakage.

The reconstruction of the real evolutionary history of the the different genera remains an unsolved problem.

Defining the genera of the eutardigrades, I indicate the shape of the apophyses for the insertion of the stylet muscles, and it is evident that until now, I have only referred to the anterior portion of the primary ridge. However, it is necessary to add information about the caudal portion, if present. In *Microhypsibius*, as in many other genera, both apophyses have two thin caudal processes pointing posteriorly and laterally (Fig. 2a).

Kristensen (1982) considered both peribuccal lamellae and peribuccal papulae to be absent. Ito (1991) described *Microhypsibius japonicus* and considered small peribuccal lamellae to be present. However, Ito wrote: "the number of the lamellae cannot be counted because of their very thin structure". In the specimens of *Microhypsibius truncatus, Microhypsibius minimus,* and *Microhypsibius bertolanii* I examined, I was not able to see peribuccal lamellae or peribuccal papulae; I did not examined specimens of *Microhypsibius japonicus* and, therefore, the presence or absence of peribuccal lamellae or peribuccal papulae cannot be confirmed.

In conclusion, the genus Microhypsibius can be defined as follows:

Diagnosis. Microhypsibiidae; cephalic elliptical organ absent; buccal tube rigid; ventral lamina absent; apophyses for the insertion of the stylet muscles asymmetrical with respect to the frontal plane; ventral apophysis in the shape of a ridge with an evident "blunt hook"; dorsal apophysis split into two portions: the anterior in shape of "semilunar hook"; the caudal portion is a little, short, thickening. Both the dorsal and ventral apophyses with two very slender caudal processes pointing posteriorly and laterally. Peribuccal lamellae and peribuccal papulae absent (?); pharyngeal apophyses and placoids present; the two branches of the furcae of the stylets have thickened, swollen and rounded apices. Lunulae absent in the known species. Smooth eggs laid in the exuviae.

Type species: Microhypsibius truncatus Thulin, 1928.

Other species: Microhypsibius minimus Kristensen, 1982, Microhypsibius bertolanii Kristensen, 1982, and probably Microhypsibius japonicus Ito, 1991.

Fractonotus, gen. nov.

Calohypsibius ornatus (Richters, 1900) is a species of Calohypsibiidae widely variable as regards the cuticular ornamentations. Pilato (1989) suggested that under the name *Calohypsibius ornatus*, "various species, and perhaps genera, are gathered". Pilato, Claxton & Binda (1989) compared *Calohypsibius ornatus* (Richters, 1900) *caelatus* (Marcus, 1928) with the typical form and with *Calohypsibius ornatus carpaticus* (Bartos, 1940). These authors stated that to *Calohypsibius ornatus caelatus* must be attributed the value of bona species named *Calohypsibius caelatus* (Marcus, 1928). I studied this species again and the analysis of the claws demonstrated that they are not of *Calohypsibius* type but of *Microhypsibius* type (Fig. 1e). Therefore, this species must be transferred to the family Microhypsibiidae.

Because of the presence of a paired elliptical organ on the head, and the different shape of the apophyses for the insertion of the stylet muscles, *Calohypsibius caelatus* cannot be ascribed to the genus *Microhypsibius*, and a new genus has to be erected to accomodate it. In reference to the shape of the dorsal apophyses for the insertion of the stylet muscles I name it *Fractonotus*.

Diagnosis. Microhypsibiidae; paired elliptical organ present on the head; buccal tube rigid; ventral lamina absent. Dorsal and ventral apophyses for the insertion of the stylet muscles asymmetrical with respect to the frontal plane; the dorsal apophysis split into two clearly distinct portions (Figs 2c, d): the anterior portion is a stumpy hook with a blunt caudal apex, the caudal portion is a longitudinal thickening. The ventral apophysis (Fig. 2c) is a very slightly prominent ridge with no hook. Both the dorsal and ventral apophyses with two very slender caudal processes pointing posteriorly and later-



Fig. 2. Bucco-pharyngeal apparatus. a. *Microhypsibius minimus* Kristensen, 1982 in dorsal view. b. *Microhypsibius bertolanii* Kristensen, 1982 in lateral view. c. *Fractonotus caelatus* (Marcus, 1928) in lateral view. d. *Fractonotus caelatus* (Marcus, 1928) in dorsal view. cp: caudal processes; da: dorsal apophysis; dlt: dorsal longitudinal thickening; va: ventral apopohysis. Scale bar = 10 μ m.

ally. Peribuccal lamellae and peribuccal papulae apparently absent. Posterior to the stylet supports, the lateral walls of the buccal tube have (Fig. 2d) a longitudinal thickening similar to that present in the genus *Ramazzottius*. Pharyngeal apophyses and placoids are present. The two branches of the furcae of the stylets have thickened, swollen and rounded apices. Lunulae absent in the known species. Smooth eggs laid in the exuviae.

Type species: Calohypsibius ornatus (Richters 1900) caelatus (Marcus, 1928).

Acknowledgements

I am grateful to Prof. R. M. Kristensen (University of Copenhagen) and Prof. R. Bertolani (University of Modena) who sent me specimens of *Microhypsibius*. I thank also Dr. Kinchin (27 Woodland Road, Slyfield Green, Guildford, Surrey, UK) for improvements of the English text.

Research supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica (60 %).

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